**3811-Pos Board B539**
**TRPM3 - A Promising Target for Analgesic Treatment**
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According to the American Pain Society, pain is one of the most common symptoms why patients search medical attention. A recent achievement in pain research was the identification of Transient Receptor Potential (TRP) channels as analgesic targets. The superfamily of TRP ion channels consists of 28 different members in mammalians. The sensitivity of TRP channels to a broad array of stimuli allows them to function as biological sensors involved in processes ranging from vision to taste, and tactile sensation. The so-called thermoTRPs (temperature sensitive) are typically expressed in sensory neurons, where they act as primary thermosensors for the detection of innocuous and noxious temperatures. Recently, our research group identified high TRPM3 expression in nociceptor neurons, where it plays a decisive role in the nocifensive response to pregnenolone sulphate (PS) and heat and in the development of heat hyperalgesia during inflammation.
This project aims to validate TRPM3 as a potential target for the development of new analgesics. Therefore, we purpose to identify new potent and selective TRPM3 blockers, and show their ability to cure different pain conditions in vivo.
In collaboration with the Center for Drug Design and Development (CD3) we identified a new potent TRPM3 blocker (CIM056741) that showed a high selectivity for TRPM3 compared to other related TRP channels. The blocker was able to inhibit PS induced $[Ca^{2+}]i$ signals in isolated DRG neurons. Furthermore, CIM056741 significantly reduced the sensitivity of mice and rats to noxious heat and PS-induced chemical pain. Interestingly, injection of CIM056741 strongly reduced the inflammatory induced mechanical hyperalgesia and was without effect on body core temperature, heart rate and locomotor activity.
In conclusion, the obtained results validate TRPM3 as a potential target for new analgesic treatments in humans.

**3812-Pos Board B540**
**Extracellular Loops are Essential for the Assembly and Function of TRPP/PKD Complexes**
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Transient receptor potential channel polycystin subfamily (TRPP) proteins assemble with polycystic kidney disease (PKD) proteins to form functionally important complexes. For example, the TRPP2/PKD1 receptor-ion channel complex plays a critical role in renal physiology. Mutations in either protein cause autosomal dominant polycystic kidney disease (ADPKD), one of the most common genetic diseases in humans. A similar complex, assembled by TRPP3 and PKD1L3, is a candidate for the sour taste receptor. The TRPP2/PKD1 complex contains three TRPP2 subunits and one PKD1 subunit and the interaction between their C-termini is crucial for the complex assembly. The TRPP3/PKD1L3 complex has the same subunit stoichiometry but its assembly involves interactions between the transmembrane segments of both proteins. These interactions have been shown to be essential for the assembly, surface expression and function of the complexes. Here we find another novel binding site between these proteins. When co-expressed in HEK293T cells, the extracellular loops between the first and second transmembrane segments (I-II loop) of TRPP2 and TRPP3 associate with the extracellular loops between the sixth and seventh transmembrane segments (VI-VII loop) of PKD1 and PKD1L3 respectively. These loops can also associate with their binding partners when the latter is expressed as full-length proteins. The loop-loop associations are functionally crucial since expression of either the TRPP3 I-II loop or the PKD1L3 VI-VII loop shows dominate negative effect on the acid-induced current of TRPP3/PKD1L3 complex. These results demonstrate, for the first time, the previously unknown essential role that these extracellular loops play in the assembly of TRPP/PKD complexes.

**3813-Pos Board B541**
**Nicotinic Acid Activates the Capsaicin Receptor TRPV1 - A Potential Mechanism for Cutaneous Flushing**
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Nicotinic acid (niacin or Vitamin B3) has been widely used in the last 50 years to treat dyslipidemias and represents an effective and safe means to reduce the risk of mortality from cardiovascular disease. Nonetheless, a substantial fraction of patients discontinue treatment due to a strong side effect of cutaneous vasodilation, commonly termed flushing. As a multi-component complex biological event, the molecular mechanism of flushing is not completely understood yet. In the present study, we tested the hypothesis that nicotinic acid causes flushing by activating the capsaicin receptor TRPV1, a polymodal cellular sensor that mediates a similar flushing response upon consumption of spicy food.
Indeed, we observed that the nicotinic acid-induced increase in blood flow was substantially reduced in $Trpv1^{-/-}$ knockout mice, indicating involvement of the channel in flushing response. Using exogenously expressed TRPV1, we confirmed that, at millimolar concentrations, nicotinic acid directly and potently activates TRPV1 from the intracellular side, but very weakly activates its homolog TRPV3 while inhibiting TRPV2 and TRPV4. Binding of nicotinic acid to TRPV1 lowers its activation threshold for heat, causing channel opening at physiological temperatures. Activation of TRPV1 by voltage or ligands (capsaicin and 2-APB) is also potentiated by nicotinic acid. Furthermore, nicotinic acid does not compete directly with capsaicin but may activate TRPV1 through the 2-APB activation pathway. Using live-cell fluorescence imaging, we observed that nicotinic acid can quickly enter the cell through a transporter-mediated pathway to activate TRPV1. In conclusion, direct activation of TRPV1 by nicotinic acid may lead to a cutaneous vasodilatory response that contributes to flushing, suggesting a potential novel pathway to inhibit flushing and improve compliance.

**3814-Pos Board B542**
**Effects of TRPM7 Inhibitors on Physiological $Mg^{2+}$ Influx in Rat Ventricular Myocytes**
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We measured free $Mg^{2+}$ concentration ($[Mg^{2+}]_i$) in rat ventricular myocytes using a fluorescent indicator furaptra (mag-fura-2). $[Mg^{2+}]_i$ decreased from ~0.9 mM to 0.2-0.5 mM by incubation of the cells in a high-$K^+$ ($Ca^{2+}$- and $Mg^{2+}$-free) solution, and recovered by perfusion with $Ca^{2+}$-free Tyrode's solution containing 1 mM $Mg^{2+}$. The time course of the $[Mg^{2+}]_i$ recovery was fitted by a single exponential function, and the first derivative at time 0 was analyzed as an initial $Mg^{2+}$ influx rate. In order to characterize physiological $Mg^{2+}$ influx pathways, we used known TRPM7 inhibitors, 2-Aminoethoxydiphenyl borate (2-APB) and NS8593. The initial rate of $Mg^{2+}$ influx was decreased to $43 \pm 10$ % (n=6) by 100 μM 2-APB, and to $12 \pm 8.6$ % (n=5) by 10 μM NS8593. These compounds inhibited the $Mg^{2+}$ influx with half inhibitory concentrations ($IC_{50}$) of 17 μM (2-APB) and 2.0 μM (NS8593). 2-APB and NS8593 also inhibited $Ni^{2+}$ influx when estimated by quenching of furaptra fluorescence with $IC_{50}$ values of, respectively, 20 μM and 4.4 μM; these values are comparable to those for $Mg^{2+}$ influx. Under the whole-cell patch-clamp configuration, removal of intracellular and extracellular divalent cations induced large inward and outward currents, $I_{MIC}$, carried by monovalent cations likely via TRPM7 channels. The $I_{MIC}$ measured at −120 mV was diminished to $48 \pm 3.6$ % (n=7) by 100 μM 2-APB, and to $50 \pm 12$ % (n=4) by 10 μM NS8593. These results support our previous conclusion [Biophys J 102:664a, 2012] that TRPM7/MIC channels serve as a major physiological pathway of $Mg^{2+}$ influx in rat ventricular myocytes.

**3815-Pos Board B543**
**Localization and Role of Transient Receptor Potential Cation Channels in Rabbit Ventricular Myocytes**
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Several members of the transient receptor potential cation (TRPC) channel family are stretch activated. They are thought to play a critical role in mechano-electrical coupling in cardiac myocytes and in cardiac hypertrophic remodeling. However, studies on their subcellular localization in cardiomyocytes are conflicting, and their functions in cardiac myocytes are barely understood. In this study, we investigated the spatial distribution of TRPC channels in isolated ventricular myocytes from adult rabbit using immunolabeling and three-dimensional confocal microscopy. Colocalization of TRPC with (i) sarcolemma labeled with wheat germ agglutinin (WGA), (ii) sarcoplasmic reticulum (SR) labeled for SR calcium ATPase (SERCA2), and (iii) cytoskeletal proteins including sarcomeric α-actinin, desmin, vinculin and β-tubulin, was assessed by a quantitative approach. Furthermore, we measured stretch-activated current with whole-cell voltage clamping. Our results from confocal imaging revealed a transverse striated distribution of TRPC1 and TRPC6 channels. We found colocalization of TRPC1 and TRPC6 with sarcomeric α-actinin, SERCA2A and desmin, but not with

WGA, vinculin and β-tubulin. Additionally, no stretch-activated current was detected when stretching isolated cardiomyocytes to approximately 10% of their resting length. Taken together, our data indicate that TRPC1 and TRPC6 are not located in the sarcolemma, but in the membrane of the SR adjacent to the Z-disks in rabbit ventricular myocytes. We suggest that a potential role of TRPC1 and TRPC6 channels is in stretch-dependent calcium leak from the SR and/or counter current balancing calcium release from the SR through ryanodine receptors.

**3816-Pos Board B544**

**Single Molecule Optical Recordings of TRPV1 Mobility and Activity**

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Inflammatory signals increase the excitability of TRPV1-expressing nociceptors, at least in part, by increasing the number TRPV1 channels in the plasma membrane (PM) of nociceptors. However, the dynamics of TRPV1 in the PM have not been studied.

Combining TIRF microscopy with whole-cell patch clamp of isolated mouse sensory neurons and HEK293T/17 cells allowed us to image both the localization of single TRPV1 molecules within cells (TRPV1-eGFP) and their activity (capsaicin-activated fluorescent "sparklets" at sites of $Ca^{2+}$ influx). Our single-molecule approach revealed the following: (1) TRPV1 channels in isolated sensory neurons and in cultured cells exhibit high lateral mobility; (2) sparklet activity elicited by capsaicin in isolated sensory neurons reveal that TRPV1 is free to move laterally in the plasma membrane while conducting $Ca^{2+}$ into the cytosol; (3) Two-state sparklet fluorescence and photobleaching analysis indicate that TRPV1 activity is not contingent on the formation of higher order structures; (4) the lateral mobility of TRPV1 in the plasma membrane decreased as a function of open duration; and (5) after addition of capsaicin, influx of $Ca^{2+}$ but not $Na^{+}$ slowed the ensemble mobility of TRPV1.

Although the mechanism by which TRPV1 activity and TRPV1 mobility are coupled and the role of mobility changes in cell signaling remain to be determined, our data demonstrate the power of single-molecule measurements to reveal aspects of signaling not observable in macroscopic experiments. Our data suggest that changes to the dynamic localization of TRPV1 by its activation may constitute a new form of regulation.

**3817-Pos Board B545**

**TRPC3 Modulates Association of Orai1 with Immunophilin FKBP12 and Orai-Mediated $Ca^{2+}$-Transcription Coupling in Mast Cells**

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Various $Ca^{2+}$-permeable channels were demonstrated to associate with immunophilins (FKBP12) as well as calcineurin in a wide range of cellular systems. A possible task of such signalosomes may be efficient translation of local calcium signals into control of gene expression. In mast cells, transcriptionally relevant $Ca^{2+}$-entry is mainly mediated by store-operated Orai channels or by the canonical transient receptor potential channel (TRPC) family. However, as yet little is known as to how these $Ca^{2+}$-permeable channels interact with FKBP12 and calcineurin and how they contribute to NFAT signaling in mast cells.

We investigated the role of TRPC3 and Orai1 channels in NFAT signaling of RBL-2H3 mast cells using electrophysiology in combination with TIRF/FRET fluorescence microscopy and heterologous expression of mutant channel proteins as well as genetic knockdown by siRNA. $Ca^{2+}$ entry via Orai1 but not TRPC3 was required for store depletion-induced NFAT activation, as indicated by the lack of effect observed after expression of a dominant negative, pore-dead TRPC3 mutant (TRPC3E630K) as well as siRNA knock-down of TRPC3. Nonetheless, overexpression of the STIM1 binding deficient mutant TRPC3D698/699K or of FKBP12 binding deficient TRPC3P704Q substantially inhibited Orai1-dependent NFAT translocation in RBL-2H3 cells. Association of TRPC3 with FKBP12 was demonstrated by TIRF/FRET microscopy and the FRET signal was strongly reduced by replacing TRPC3 by TRPC3P704Q. Interestingly, association of Orai1 into FKBP12-containing signalosomes was similarly observed in TIRF/FRET experiments, and overexpression of TRPC3 facilitated the association of Orai1 with FKBP12 as well as calcineurin. Moreover, these interactions were reduced by overexpression of TRPC3P704Q.

From our data we conclude that TRPC3 is a structural element of an immunophilin comprising protein complex in mast cells, thereby facilitating the interaction of Orai1 with FKBP12/calcineurin and NFAT signaling.

**3818-Pos Board B546**

**$Ca^{2+}$ Facilitates TRPC4 Activation by Gi/O Signaling in Both Calmodulin Dependent and Independent Manner**

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TRPC4 is a member of the Canonical Transient Receptor Potential family of non-selective ion channels and is regulated by a set of intracellular and extracellular factors including G protein coupled receptor activation, calcium and membrane phospholipids. Sodium ($Na^{+}$) and calcium ($Ca^{2+}$) influx through the active TRPC4 channel elicits membrane depolarization and intracellular $Ca^{2+}$ signaling in neurons, vascular endothelium and smooth muscle cells. Uniquely, TRPC4 is maximally activated only when two separate G protein pathways, $G_{q/11}$ and $G_{i/o}$, are co-stimulated, making it a coincidence detector of $G_{q/11}$- and $G_{i/o}$-coupled receptor co-activation. This function may be important for coordinating the actions of sympathetic (e.g. adrenergic) and parasympathetic (e.g. cholinergic) innervations. While a direct interaction between $G_{i/o\text{-}alpha}$ subunits and the cytoplasmic C-terminal domain of TRPC4 has been suggested to underlie the activation mechanism by $G_{i/o}$ proteins, the PLC-$IP_3$-$IP_3R$ pathway is thought to be critical for the action of $G_{q/11}$. Using electrophysiological recordings of HEK293 cells heterologously expressing mouse TRPC4-beta, we examined the interdependence of $G_{i/o}$ and $G_{q/11}$ pathways on TRPC4 activation. We show that whereas $G_{i/o}$ (but not $G_{q/11}$) stimulation is necessary, it does not fully activate TRPC4 unless accompanied by a simultaneous rise in intracellular free $Ca^{2+}$, which may or may not require activation of the $G_{q/11}$ pathway. Paradoxically, inhibiting calmodulin also facilitated TRPC4 activation by $G_{i/o}$ signaling, but not to the full extent as that facilitated by increasing intracellular $Ca^{2+}$ levels, indicating an inhibitory role played by calmodulin on TRPC4 activation and a calmodulin-independent positive regulation by $Ca^{2+}$. Our findings indicate that TRPC4 is a coincidence sensor of (A) $G_{i/o}$ stimulation and (B) intracellular [$Ca^{2+}$] rise that can be triggered by receptor-PLC pathways, lysosomal $Ca^{2+}$ release, ER $Ca^{2+}$ store release and plasma membrane $Ca^{2+}$ influx mechanisms.

**3819-Pos Board B547**

**Exploring the Architecture of the Outer Pore of the TRPV1 Channel with Double-Knot Toxin**

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The TRPV1 channel is a non-selective homotetrameric cation channel that acts in nociceptors as a sensor for external noxious chemicals and physical stimuli. It is also modulated by many cell-signaling molecules whose concentrations increase during inflammation and tissue damage. As a consequence, its function has been associated with inflammatory hyperalgesia and neuropatic pain. At present, little structural or mechanistic information is available for this protein. However, the pore domain is thought to play a fundamental role in channel function, since modulating signals are expected to converge on the pore to gate ion permeation. There is also growing evidence to suggest that the pore is the site of action of protons, possibly temperature, and the double ICK knot tarantula toxin (DkTx). DkTx, or its isolated single knots, have been proposed to bind to the external pore to promote activation. Here, we set out to study DkTx-TRPV1 interactions to gain structural information on the pore domain. By using a concatenated TRPV1 channel tetramer, in which toxin-activation was disrupted in specific subunits, we found that the binding of a single toxin knot is sufficient to promote channel activation. We also found that DkTx activates channels in which toxin-activation has been disrupted in either adjacent or opposite subunits, indicating that the knots of DkTx can arrange differently to activate the TRPV1 channel. Finally, we found that a hexa-histidine tag attached to the N-terminus of the toxin acts a voltage- and pH-dependent blocker. We are currently using variants of DkTx, in combination with concatenated TRPV1 channels, to delineate the toxin binding site on the external surface of the channel relative to the central pore and to probe agonist-dependent conformational changes in the pore.